TECHNICAL REPORT
NATICK/TR-03/029

AD________________

SPINNING OF FIBERS FROM AQUEOUS SOLUTIONS

by
Steven Arcidiacono

August 2003

Final Report
October 2000 – September 2002

Approved for public release; distribution is unlimited

U.S. Army Soldier and Biological Chemical Command
Soldier Systems Center
Natick, Massachusetts 01760-5018

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
11-8-03	Final	Oct 2000-Sept 2002

4. TITLE AND SUBTITLE	
SPINNING OF FIBERS FROM AQUEOUS SOLUTIONS	5a. CONTRACT NUMBER
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S) Steven Arcidiacono	5d. PROJECT NUMBER 300509
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) US Army Soldier and Biological Chemical Command Soldier Systems Center ATTN: AMSSB-RCF-P(N) Natick, MA 01760-5018	8. PERFORMING ORGANIZATION REPORT NUMBER NATICK/TR-03/029
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) US Army Armament Research, Development, and Engineering Center ATTN: AMSTA_AR_DB, BLDG 1 Picatinney Arsenal, NJ 07806-5000	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release:distribution is unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Previous funding supporting this research focused primarily on development of the aqueous-based method for processing silk into spin solutions. Much of this effort consisted of production of recombinant silk protein in bacterial and yeast expression systems. In spite of the small quantities produced and silk solublity problems, a method for spinning silk fibers under aqueous conditions was developed. However, these fibers were of poor quality and could not be characterized by mechanical tensile testing. Orientation of the protein along the fiber axis was observed by polarizing light microscopy. This was only qualitative, however, and quantitative measurement (e.g., mechanical testing) was needed for fiber evaluation. Under a Cooperative Research and Development Agreement, larger quantities of recombinant silk protein provided by Nexia Biotechnologies were processed for fiber spinning. Spin solutions with protein concentrations as high as 28% were achieved and spun into fibers with measurable mechanical properties similar to natural spider silk. The larger quantities of silk allowed for the examination of the parameters in spin dope preparation, fiber spinning and post spinning treatment to begin to be explored.

15. SUBJECT TERMS

SILK	MECHANICAL PROPERTIES	RENEWABLE RESOURCES
FIBERS	SPINNING(INDUSTRIAL PROCESSES)	HIGH PERFORMANCE
AQUEOUS SOLUTIONS	ENVIRONMENTALLY FRIENDLY	RECOMBINANT

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			S. Arcidiacono
U	U	U	UU	22	19b. TELEPHONE NUMBER *(Include area code)* (508) 233-4983

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PREFACE

The U.S. Army Natick Soldier Systems Center, Soldier and Biological Chemical Command, Natick, MA, prepared silk solutions for fiber spinning and subsequent mechanical testing. Fibers were spun under aqueous conditions that had properties similar to natural spider silk. Solution processing, and spinning variables and post spinning treatments were all found to influence fiber mechanical properties.

This research was undertaken with funding under the Environmental Quality Basic Research (EQBR) program. The project was conducted from 1 October 2000 to 30 September 2002 under contract EQBR project #BH67, and under Natick project number 300509.

Natick staff members participating in this research included:

Steve Arcidiacono (POC), microbiologist;
Phone: (508) 233-4983, DSN 256-4983
FAX: (508) 233-5521
email: arcidiaconos@natick.army.mil

Jason Soares: chemical engineer

Elizabeth Welsh: research chemist

Charlene Mello: research chemist

Diane Steeves: research chemist

ACKNOWLEDGEMENTS

Natick is indebted to the following research partners for their assistance in the project:

Nexia Biotechnologies Inc. (Vaudreuil-Dorion, QC Canada) - provide large quantities of recombinant silk protein to optimize processing and spinning under a Cooperative Research and Development Agreement (CRADA).

The Center to Advance Molecular Interaction Sciences (CAMIS, University of New Hampshire, Durham, NH) - biophysical characterization to examine the behavior of spin solutions.

University of California at Santa Barbara (UCSB) – analysis of silk solution for fibril formation.

SPINNING OF FIBERS FROM AQUEOUS SOLUTIONS

Executive Summary

Technical Objective: To develop methods for producing high-strength fibers from environmentally friendly aqueous solutions. Materials derived from renewable (i.e., biological) sources will reduce the use of organic or petroleum-based starting materials used in synthetic fiber manufacture. Aqueous-based processes will reduce or eliminate the use of hazardous materials and hazardous waste generation.

Environmental Problem: Current high-performance fibers used by the Army and DoD in general are produced using petroleum-based starting materials and processed in an environmentally unfriendly manner. In contrast, the use of aqueous-based materials and processes will reduce or eliminate the use of organic solvents and reduce reliance on imported petroleum. Costs will be lowered due to recycling of material and reduction in the amount of hazardous waste generated.

This proposal addresses the Army User Requirement #3.10e, Reduce/Eliminate Pollution from the Manufacturing and Testing of Military Clothing and Textiles.

Funding Profile:

FY	01	02	03
$K	250	240	Transition to 6.2

Non-EQBRD resources: Nexia Biotechnologies (scale-up production of recombinant silk protein for processing); CAMIS (solution biophysical characterization); UCSB (solution analysis)

Summary of Results:

Previous funding supporting this research focused primarily on development of the aqueous-based method for processing silk into spin solutions. Much of this effort consisted of production of recombinant silk protein, including investigation of fermentation in bacterial[1] and yeast expression systems. Due to problems within the organisms at the genetic level, only mg quantities of protein were produced. Spin solutions were also difficult to manipulate due to the unique solubility characteristics of silk, ie., maintaining solubility was a major challenge. In spite of the small quantities and the propensity of silk protein to become insoluble, we were capable of developing a method for spinning silk fibers under aqueous conditions[2]. However, these fibers were of poor quality and could not be characterized by mechanical tensile testing. Orientation of the proteins along the fiber axis was observed by polarizing light microscopy. This was only qualitative, however, and quantitative measurement (e.g., mechanical testing) was needed for fiber evaluation.

Under a Cooperative Research and Development Agreement with Nexia Biotechnologies, larger quantities of recombinant silk protein relative to what was produced in-house was obtained for processing and fiber spinning. Unlike silk solutions from bacterial fermentation silk, these solutions were able to be spun into fibers using the aqueous spinning process developed at Natick. This was an important result because the Nexia recombinant protein was based on a different silk protein than those produced in-house and also was produced using a different expression system (tissue culture vs. bacterial fermentation).

Spinning fibers that could be mechanically tested became possible when larger quantities of silk became available. Spin solutions with protein concentrations as high as 28% were achieved and spun into fibers with measurable mechanical properties[3]. This allowed for the examination of the parameters in spin dope preparation, fiber spinning and post spinning treatment to begin to be explored.

Conclusion: Fibers have been spun from recombinant silk proteins that have properties similar to natural dragline silk. We have moved from simply spinning fibers to producing fibers with desirable mechanical properties. While this is a significant contribution, many unanswered questions remain. Reproducibly spinning fibers with desired properties will entail an understanding of the impact each spinning and post-spinning variable has on fiber properties. Optimization of the spinning method and ultimately fiber properties will require larger quantities of recombinant silk protein than is currently available. Given sufficient material, the potential of spider silk as a useful biomaterial may be realized.

Introduction

Environmental Problem: Current high-performance fibers used by the Army and DoD in general are produced using petroleum-based starting materials and processed in an environmentally unfriendly manner. These materials can also be non-biodegradable and are unable to be recycled. For example, the starting material for Kevlar® is terphthaloyl chloride (produced from petroleum) and is processed with fuming sulfuric acid. DuPont® produces approximately 2 million tons of Kevlar per year, using 15,800,000 to 18,750,00 pounds of acid. Specifically, the Army uses about 10,000 pounds of Kevlar per year for composite applications. Other high performance materials such as Spectra and PBO are also composed of environmentally unfriendly precursors and require hazardous compounds for processing. In contrast, the use of aqueous-based materials and processes will reduce or eliminate the use of organic solvents and reduce reliance on imported petroleum. Costs will be lowered due to recycling of material and reduction in the amount of hazardous waste generated.

Approach: Silk polymers offer the potential to be used in applications requiring a high-performance fiber. Natural spider silk can absorb more energy before breaking than Kevlar (Figure 1), making it an attractive material to duplicate. Under previous

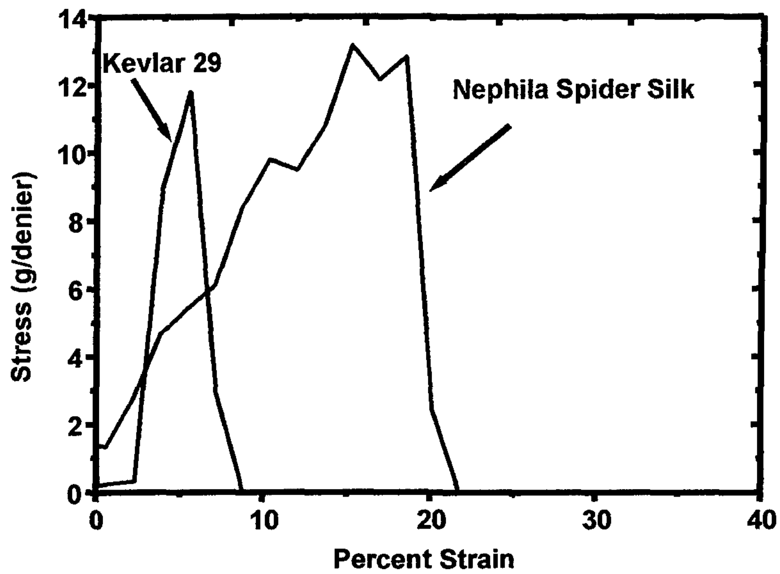


Figure 1. Stress/strain comparison of Kevlar 29 and *Nephila clavipes* dragline silk. Spider silk can absorb more energy than Kevlar 29, as represented by area under the curve.

funding, the ability to produce and process silk protein into fibers using an aqueous-based method has been demonstrated using milligram quantities of three different recombinant proteins based on spider silk produced in-house (Table 1).

Table 1. Silk proteins used for aqueous spinning. Proteins produced at Natick were suitable for developing an aqueous fiber spinning method. Silk provided by Nexia produced fibers with measurable mechanical properties and allowed identification of important variables and their effect on fiber properties.

Recombinant silk protein	**Silk gene**	**Expression system**	**Source**
NcDS	*Nephila clavipes* SpI	Bacterial Fermentation	US Army Natick Soldier Center
$[(SpI)_4/(SpII)_1]_4$	Synthetic spidoin I and II	Bacterial Fermentation	US Army Natick Soldier Center
$(SpI)_7$	Synthetic spidroin I	Bacterial Fermentation	US Army Natick Soldier Center
BS3	*Araneus diadematus* ADF-3	Tissue culture	Nexia Biotechnologies
M3	*Araneus diadematus* ADF-3	Tissue culture	Nexia Biotechnologies
M5	*Nephila clavipes*	Trangenic goats	Nexia Biotechnologies

Essentially, silk protein was produced by bacterial fermentation and purified. The purified silk solution was concentrated using ultrafiltration to reduce the sample volume by removing the dilute denaturing buffer and retaining the silk protein in a soluble state. The concentrated silk solution (at volumes as small as 25 ul) was wet spun into a methanol/water coagulation bath (Figure 2), and when possible was drawn to the maximum extent possible and collected for evaluation. Poor quality fibers were often brittle and impossible to evaluate by mechanical testing.

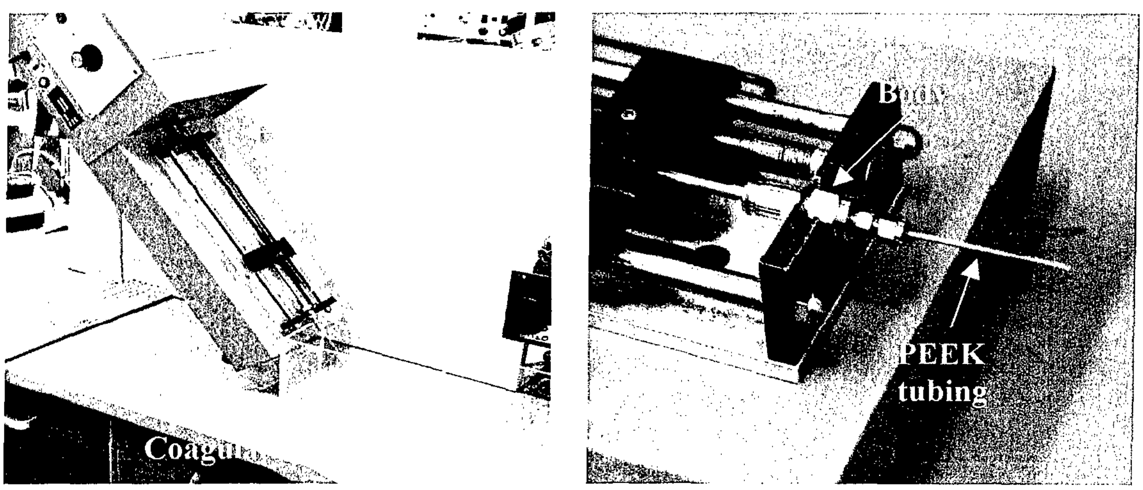


Figure 2. Microspinner used to spin silk fibers. Silk spin solution was loaded into the body of the microspinner and extruded through the 0.005" PEEK HPLC tubing into the coagulation bath, where it formed a fiber. Suitable samples were drawn and collected for mechanical testing.

The goal was to optimize the processing and fiber spinning method to obtain material with the desired mechanical and physical properties. Again, due to limitations in the amount of recombinant protein, optimization of the method did not precede much beyond assessing spinnability and some qualitative observations done by microscopy.

Processing and spin solution preparation was kept essentially the same when Nexia Biotechnologies supplied purified silk solutions. Larger quantities, (up to 1 g per batch) made it possible to investigate the variables important to optimize the fiber spinning method. Fibers were spun under a variety of conditions and tested on an Instron 5500R, Model 4201 to determine mechanical properties. Fibers were broken into a number of pieces suitable for testing (1/2" test length); the results from each test were combined to give an average set of properties for a given fiber. Fiber properties were then correlated with spinning conditions to optimize the spinning method. Producing fibers is a multivariate problem (Table 2), and important variables affecting fiber properties need to be identified before the optimal spinning conditions can be used.

Table 2. Some of the variables needing to be examined to optimize fiber properties. The importance of each variable would be determined empirically, and any interaction between variables would also need to be considered.

Spinning	Protein concentration Protein purity Age of spin solution Spin solution composition Coagulation bath composition Spin speed
Post-spinning	Draw ratio Draw speed Draw solvent composition Draw temperature

Results

Silk proteins from bacterial fermentation

Recombinant proteins produced by bacterial fermentation were capable of being spun into fibers (Table 3). The fibers had diameters from 10 to 60 um, were water insoluble and exhibited orientation as evidenced be birefringence under polarizing light microscopy. The fibers were mildly tacky when first spun and became less with time. This was dependent, however on the composition of the methanol bath; if the methanol concentration was too high, the fibers coagulated immediately and were brittle. The fibers had uniform diameters, and under scanning electron microscopy had a smooth surface.

Table 3. Summary of recombinant proteins produced by bacterial fermentation.

Protein	Size (daltons)	Protein Concentration	Fiber Formation	Fiber Diameter (um)
NcDS	42,970	25%	yes	60
$[(SpI)_4/(SpII)_1]_4$	55,731	12.5%	yes	20-31
$(SpI)_7$	24,000	35%	no	-

The spinning solutions had protein concentrations as high as 25% (w/v) with a typical purity of greater than 70%. Not all the silk solutions were capable of fiber formation; the $(SpI)_7$ protein was unable to form intact fibers, most likely due to the size of the protein. No fibers from NcDS or $[(SpI)_4/(SpII)_1]_4$ were of sufficient quality to allow determination of mechanical properties. They were either impossible to remove from the coagulation bath or too brittle and broke during preparation for mechanical testing.

Another hurdle was the difficulty maintaining protein solubility during spin solution preparation; precipitation or gelation would occur as the protein concentration increased. Solutions might undergo partial or complete gelation. If gelation was complete the sample was unable to be spun. Solutions were examined to look for conformational (2^o structure) changes similar to those observed in natural spider silk. Changes in 2^o structure of spin solutions have been observed over time, the extent of which is dependent on buffer concentration and temperature. Solutions acquired a greater β sheet content (high β sheet is characteristic of natural spider silk) depending on buffer composition. Elevated temperature accelerated β sheet formation, while the concentration of denaturant in the buffer determined the rate of β sheet formation. The presence of denaturant disrupts hydrogen bonding; a lower concentration of denaturant allows the hydrogen bond formation that is necessary for β sheet formation. Spin solutions also undergo changes other than β sheet formation. A 25% NcDS solution was unable to be spun into fibers until it had incubated for several days, and there was no significant change in 2^o structure. Additionally, dilute solutions were observed to undergo self-association, or protein-protein interaction. The meaning of these observed changes in solution characteristics was unclear, since there was no opportunity to correlate them with fiber properties.

For further details, please refer to the article "Aqueous Processing and Fiber Spinning of Recombinant Spider Silks", Arcidiacono, S., et al., Macromolecules, 2002. **35**: p. 1262-1266.

Silk proteins from tissue culture

Greater progress was achieved with recombinant silk protein provided by Nexia Biotechnologies. This supply was a great time saver because it eliminated the need for protein production by fermentation, isolation and purification. The silk solutions were in greater quantity than what could be produced in-house by bacterial fermentation and also of high purity. In addition, the fibers produced were not brittle and capable of being evaluated by mechanical tensile testing. This allowed identification of important variables and their effect on fiber properties.

A large number of fibers were generated from two recombinant proteins based on *Araneus diadematus* silk (BS3, M3) and a 3rd based on *N. clavipes* silk (M5) (Table 4). Initial studies were done with BS3, a recombinant silk containing a 6xHis affinity tag for purification. These studies showed that the protein were able to be spun into fibers using the aqueous method developed for the in-house material. Fibers of 20-40 cm in length could be produced and were water insoluble (Figure 3). Only a few samples were tested, but they were brittle and did not possess measurable mechanical properties.

Table 4. Summary of spin results of recombinant silk proteins provided be Nexia Biotechnologies. Spinning fibers form BS3 confirmed that the aqueous spinning method worked an Araneus silk protein produced in tissue culture. M3 fibers had measurable mechanical properties and allowed some important spinning variables to be identified. M5 was able to be spun into fibers but were very brittle.

Protein	Size (daltons)	Protein Concentration	Purity	Fiber Diameter (um)
BS3	60,800	2.8-11%	70-80%	8-23
M3	59,000	10-28%	80-90%	15-60
M5	54,194	18%	60-80%	13-40

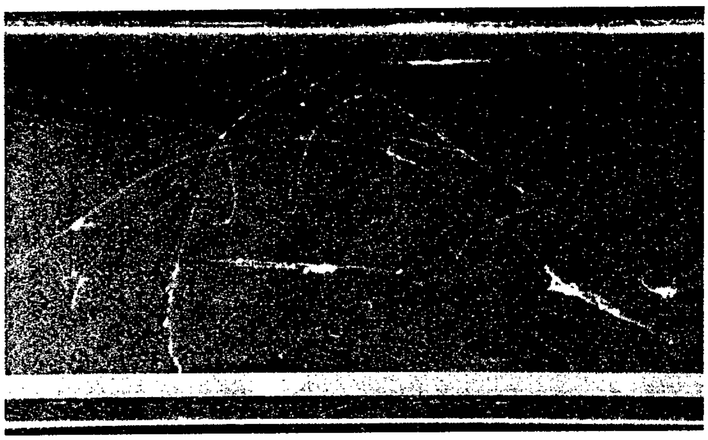
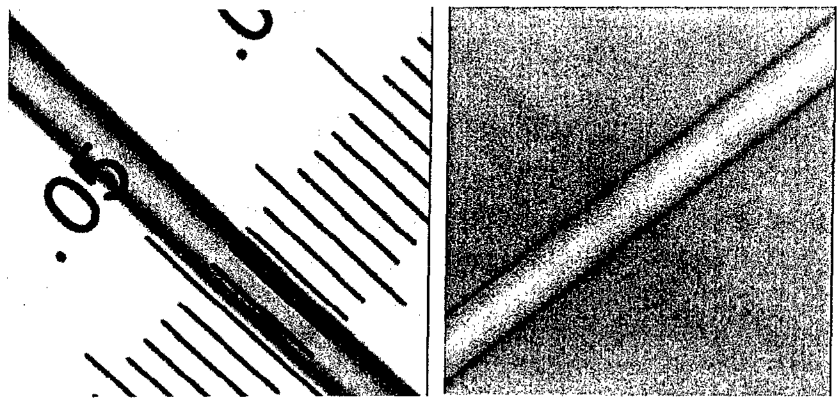


Figure 3. BS3 fibers in coagulation bath.

The presence of the 6xHis tag on BS3 was a concern because it is not naturally part of the natural silk gene and its effect on fiber formation and mechanical properties was not known. To resolve this issue, the research then focused on M3 silk solutions (same silk gene as BS3, without the 6xHis tag). Large numbers of fibers were produced due to the increased quantity of material provided. It was possible to evaluate the effect of different spinning conditions on fiber properties. The fibers were water insoluble, had uniform diameters and exhibited orientation as observed by birefringence (Figure 4).



A B

Figure 4. Light microscopy of M3 fiber from a 22% protein solution. Fiber exhibits birefringence and has an average fiber diameter of 21 um. A. Regular light B. Polarizing light with a 530 nm tint plate.

The fibers with the best properties were similar to the natural dragline silk from the spider *Araneus*, but did not duplicate it (Table 5). Toughness and modulus were similar, but the fibers had a higher % strain to break. Although the fibers could absorb as much energy as the natural silk, they would do so by stretching more before breaking.

Table 5. Comparison of mechanical properties of recombinant silk fibers with those of the native silk.

Fiber	Draw ratio	Toughness (g/den)	Modulus (g/d)	% Strain to break	Tenacity (g/d)	# samples tested
M3, sample 1	5	0.895	43	60	1.9	7
M3, sample 2	5	0.850	111	43	2.3	7
M3, sample 3	4	0.645	63	45	1.8	5
Araneus dragline	na	0.6-1.3	38-76	19-30	7-11	20

na – not applicable

Mechanical properties were affected at least in part by several variables: protein concentration and purity, coagulation bath composition, and post-spin draw. Concentration, purity and coagulation bath composition each effect fiber formation, while draw imparts molecular orientation needed for improved mechanical properties.

In general, solutions of 10% or more protein were needed to produce fibers with measurable properties. The highest tensile properties were achieved from fibers from >23% solutions that underwent post-spin draw. As-spun fibers had an average diameter of 40 um, while the average decreased to 20 um after the maximum amount of post-spin draw (5x the original length). Toughness, tenacity, and modulus all improved with increased draw. The solution in which draw took place was also important. Fibers drawn first in methanol/water and further drawn in water (double draw) had improved toughness and modulus relative to draw in methanol/water (single draw) (Figure 5).

There was a large amount of variability in fiber properties; there was fiber-to-fiber variability between fibers spun under the same conditions, and variability within the samples that comprised an entire fiber (fibers were broken into smaller lengths to generate ½ inch samples for testing). Differences between fibers might be attributed to protein concentration, uneven draw due to drawing by hand, etc. Due to hand draw, the draw rate and to a lesser extent the actual draw ratio most likely varied. It is expected that there will be inherent inconsistencies due to hand draw, which might be avoided if mechanical draw was used. Fibers of sufficient quality were produced that could be reeled mechanically from the coagulation bath. The fibers were undrawn however; despite numerous attempts we were not successful at drawing while mechanically reeling. Other post spinning factors such as length of time the fibers spent in the coagulation bath had no obvious effect on properties.

For a given fiber the variability could be large, suggesting that a fiber is not the same over its entire length (Table 6). It was observed during the drawing process that many fibers did not draw uniformly, with certain areas drawing more than others. Since all the samples are produced under identical conditions, one possible cause is that the spin solutions are not uniform at the molecular level. As stated above, silk solutions

Table 6. Mechanical data for ½ inch samples taken from an M3 fiber drawn in methanol then water, with a draw ratio of 5.

Sample	Toughness (g/d)	Modulus (g/d)	% Strain to break	Tenacity (g/d)
1	0.785	52.4	50.1	2.02
2	0.399	172.8	31.2	1.67
3	0.876	52.2	49.8	2.25
4	0.527	112.4	34.1	1.92
5	0.579	165.8	32.7	2.22
6	1.274	61.3	58.4	2.77
7	1.541	157.4	67.2	2.94
Mean	0.854	110.6	46.2	2.26
Std. Dev.	0.417	55.3	14.0	0.456

undergo conformational changes in 2° structure (formation of β sheet) and self-association (interaction between protein molecules). The changes may result in a solution of a heterogeneous population of proteins with varying conformations. Proteins that have undergone less self-assembly might form a section of fiber able to be drawn further than an area comprised of molecules that are locked in by greater protein-protein interaction. The other possibility is that fiber inconsistency was caused during spinning, such as turbulent flow of the spin solution or uneven backpressure within the microspinner body (spin solutions are somewhat viscous, but this has never been quantitated). Periodic build-up of backpressure and eventual release could affect the amount of shear occurring in the PEEK tubing, which could cause inconsistent alignment of protein in the fiber and uneven draw. Unfortunately it was not possible to monitor backpressure with the spinning apparatus.

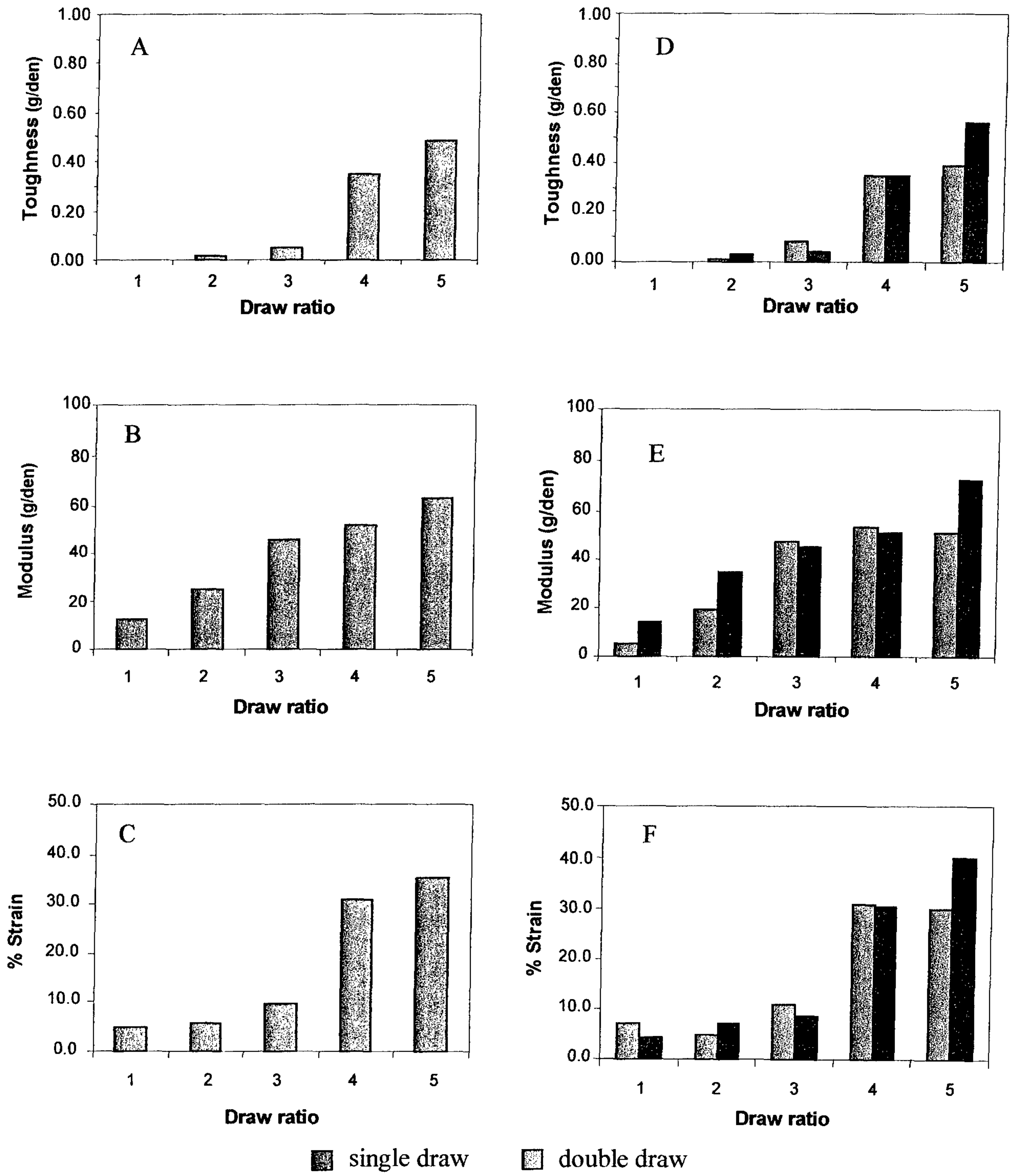
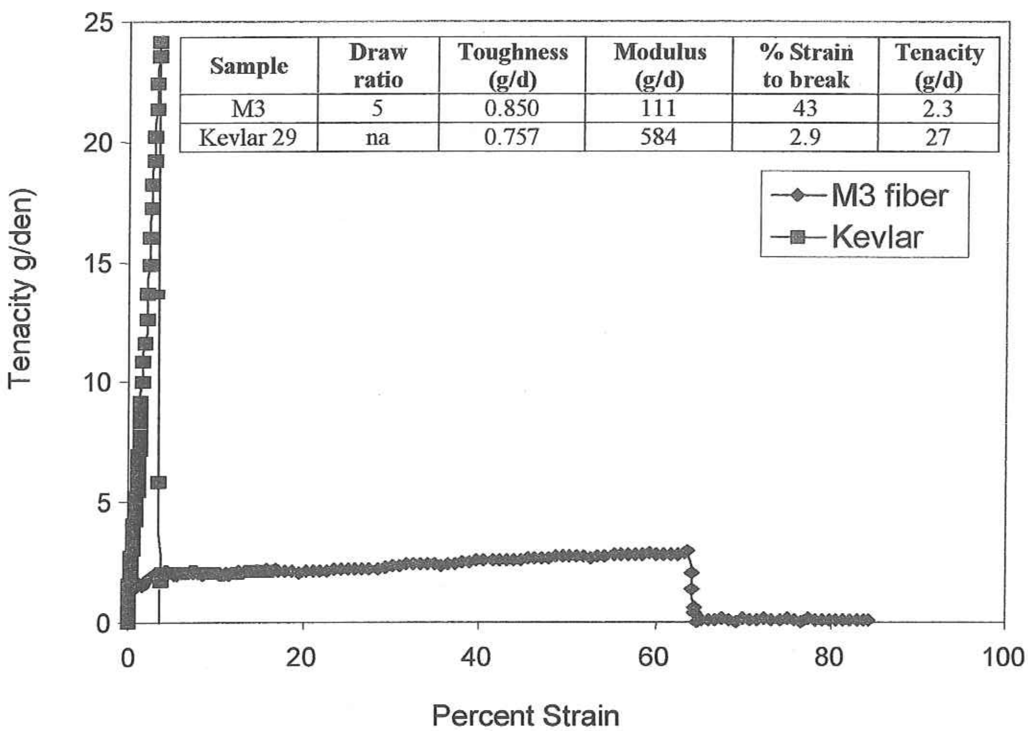


Figure 5. Effect of post-spinning draw on toughness (A and D), modulus (B and E), and % strain (C and F) to break in M3 fibers. A, B, C are averages of all fiber properties at different draw ratios. D, E, and F compare effect of single and double draw on fibers. Increasing draw improves mechanical properties by orienting the silk molecules along the fiber axis.

As promising as these results are, the best mechanical properties achieved thus far would not be considered characteristic of a high-performance fiber. Fibers such as Kevlar 29 exhibit good toughness without a high strain to break. Although the toughness of the recombinant fibers is comparable to Kevlar, they are far too stretchy (ie., they elongate too much before breaking) (Figure 6). It is possible that this amount of elongation could be reduced (resulting in a corresponding increase in the modulus, or stiffness) if the draw ratio



Sample	Draw ratio	Toughness (g/d)	Modulus (g/d)	% Strain to break	Tenacity (g/d)
M3	5	0.850	111	43	2.3
Kevlar 29	na	0.757	584	2.9	27

Figure 6. Comparison of mechanical properties of Kevlar to M3 fiber. Although the fibers have similar toughness, the M3 toughness is due to its high strain to break.

was optimized, resulting in an improvement of properties. There is evidence that fiber draw has not been optimized as observed by microscopy (Figure 7) in which the fiber experienced additional draw during tensile testing.

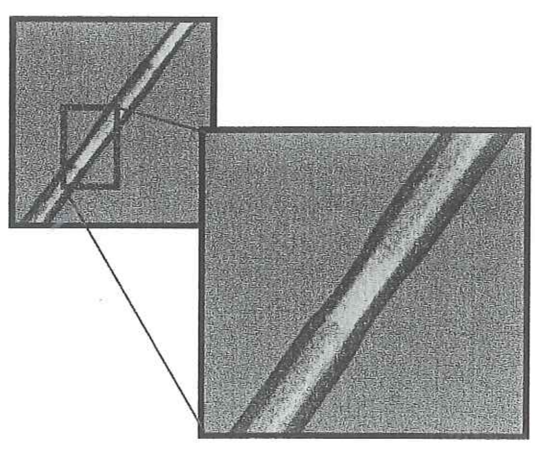


Figure 7. Fiber spun from a 22% solution. Fiber was double drawn in methanol and then water for a total draw ratio of 4. Areas of elongation occur after fiber has undergone mechanical testing.

Other possibilities for increasing the modulus is incorporate a material known to improve tensile strength, such as nanoclay. Preliminary studies of the effect of nanoclay were done by adding 3% clay to a 17% M3 spin solution, but there was no improvement in fiber properties. However, due to the small volumes used there may not have been sufficient mixing for the clay to have an effect (complete dispersion is needed to prevent clumping of the clay, which would decrease mechanical properties).

Additional details can be found in the article "Spider Silk Fibers Spun from Soluble recombinant Silk Produced in Mammalian Epithelial Cells", Lazaris, A., et al., Science, 2002. **295**(5554): p. 472-476. The following web site has additional information on this publication: http://www.sciencemag.org/cgi/content/full/295/5554/472/DC1

Silk protein from transgenic goats

Additional research is needed to optimize the spin method by identifying variables (individually and in combination) that affect fiber properties. This requires additional material, which may be possible by using transgenic expression systems, proven to produce kg quantities of other recombinant proteins. Nexia has supplied the recombinant silk protein M5 in (relatively) large quantity (approximately 1g) produced in goats for fiber spinning. Preliminary results showed that the protein could be spun and the fibers capable of being drawn (Figure 8). In contrast to the M3 fibers, many of the fibers had a rough surface and inconsistent diameter. The protein was not as pure as we would have liked, and there were also issues with maintaining solubility at higher concentrations (>20%). The fibers were brittle and were difficult to test for mechanical properties. The batches of material supplied to us were among the first Nexia produced and the purification, etc. was still being optimized. Also, the silk protein was different than M3, as well as the expression system and processing method, all of which could have contributed to the difficulties experienced. Unfortunately, these samples were studied during the last year of funding and could not be examined further.

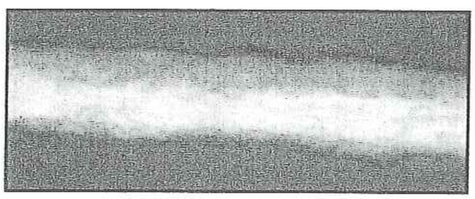


Figure 8. M5 fiber from an 18% spin solution under polarizing light microscopy. Fibers could be drawn up to a draw ratio of 4. The fibers were brittle and did not have good mechanical properties.

Conclusion

The ability to spin recombinant silk fibers similar to that of natural spider silk using aqueous solutions has been demonstrated. Once optimized, it offers the possibility of producing high-performance fibers while avoiding harsh and environmentally unfriendly methods used in synthetic fiber production. While spinning recombinant silk fibers with measurable mechanical properties is a significant achievement, the fiber properties do not duplicate nature silk. There is also an undesirable amount of variability in the mechanical properties because the spinning method is not yet optimized. The goal of the project was to replace synthetic high-performance fibers made from hazardous materials or processes, such as Kevlar. While the recombinant fibers exhibit good properties, the amount of elongation prior to breaking would not be consistent with the properties of a high-performance fiber. Additional research is needed to optimize the spin method by identifying variables (individually and in combination) that affect fiber properties. This requires additional material, which may be possible by using transgenic expression systems, proven to produce kg quantities of other recombinant proteins. Nexia Biotechnologies is producing silk in transgenic goats and is working with a leading international medical textile company Acordis Speciality Fibres, Ltd. (United Kingdom) to commercialize the recombinant silk product BioSteel.

Publications, patents and presentations

1. Arcidiacono, S., et al., *Purification and characterization of recombinant spider silk expressed in Escherichia coli.* Applied Microbiology and Biotechnology, 1998. **49**: p. 31-38.
2. Arcidiacono, S., et al., *Aqueous Processing and Fiber Spinning of Recombinant Spider Silks.* Macromolecules, 2002. **35**: p. 1262-1266.
3. Lazaris, A., et al., *Spider Silk Fibers Spun from Soluble recombinant Silk Produced in Mammalian Epithelial Cells.* Science, 2002. **295**(5554): p. 472-476.
4. Arcidiacono, S., M.M. Butler, and C.M. Mello, *A Rapid Selective Extraction Procedure for the Outer Membrane Protein (OmpF) from Escherichia coli.* Protein Expression and Purification, 2002. **25**: p. 134-137.
5. Oroudjev, E., et al., *Segmented nanofibers of spider dragline silk: atomic force microscopy and single-molecule force spectroscopy.* Proc Natl Acad Sci U S A., 2002. **99**: p. 6460-5.

US patent
Methods for the Purification and Aqueous Fiber Spinning of Recombinant Spider Silks and Other Structural Proteins US 09/490,291 – allowed and awaiting issue

International Patent
Methods for the Purification and Aqueous Fiber Spinning of Recombinant Spider Silks and Other Structural Proteins International Application PCT/US00/30086

April 2002 Presented "Spinning Recombinant Spider Silk: Aqueous Processing of Fibers" at the national Materials Research Society meeting.

November 2001 Presented "Aqueous Processing of Fibers", to the Annual Picatinny Arsenal Chapter of the National Defense Industrial Association (NDIA).

November 2000 Presented "Recombinant Spider Silk Fibers" at the 2nd Annual Non-lethal Technology and Academic Research (NTAR) Symposium.

This document reports research undertaken at the U.S. Army Soldier and Biological Chemical Command, Soldier Systems Center, Natick, MA, and has been assigned No. NATICK/TR-03/029 in a series of reports approved for publication.